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Studies on the Uptake, Metabolism, and Release of Endogenous and Exogenous Chemicals by Use of the Isolated Perfused Lung

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The isolated perfused lung is a valuable tool for studying many lung functions. The kinds of information one can obtain from the isolated perfused lung are illustrated by examples from our studies on the uptake, accumulation, and metabolism of endogenous and exogenous chemicals.

In recent years it has been recognized that the lung possesses a number of important functions other than serving as an organ for the exchange of gases. It has often been assumed that the lung plays only a passive role in the removal and metabolism of various chemicals from the circulation. Today it is recognized that the lung serves as a filter for the blood, removing and detoxifying various endogenous substances; for example, 5-hydroxytryptamine (5-HT), norepinephrine (NE), and prostaglandin (PG) (*1*) and in other cases activating endogenous substances, e.g., angiotensin. Under normal conditions or under stress, the lung serves as an endocrine organ releasing vasoactive material into the circulation (*1*). In addition, the lung possesses the ability to metabolize exogenous chemicals (*2*). Since the lung is in direct contact with the environment, toxicity produced by environmental chemicals may be related to these nonrespiratory functions.

Many studies on the nonrespiratory lung functions have been made possible or aided by the isolated perfused lung preparation (IPL). We have used this isolated perfused rat and rabbit lung preparation to examine the uptake and metabolism of various chemicals. Figure 1 illustrates our IPL, which is a modification of the basic lung perfusion technique developed by Niemeyer and Bingham (*3*). Our modified perfusion apparatus can be used in three ways: (1) the perfusate or blood is recirculated, and the uptake and metabolism of a chemical by the lung is investigated by examination of the changes in the perfusate concentrations with time; (2) the perfusate is not recirculated (single pass), the labeled test compounds and labeled vascular maker dextran being injected as a bolus into the pulmonary artery and the effluent collected as a series of timed samples; (3) the perfusate is not recirculated, but lungs being perfused with "chemical-free" media are then perfused with perfusate containing the test compound. Effluent from the lung is collected as a series of timed samples during this time. In addition, the efflux of accumulated chemicals from the lung can then be studied by perfusing with "chemical-free" perfusate.

From each of these types of experiments one can obtain a variety of information concerning the uptake, accumulation, release and metabolism of

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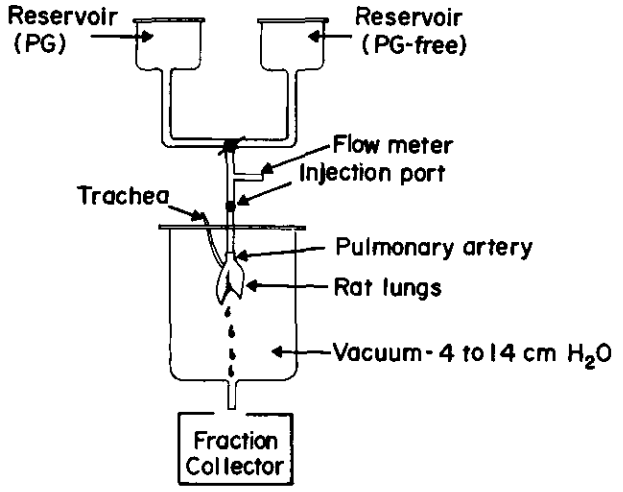

FIGURE 1. Schematic diagram of isolated perfused lung (IPL).

chemicals from the lung. We would like to illustrate this point using an example from our work.

We have studied the accumulation and metabolism of a number of xenobiotics by the recirculating rabbit IPL (*4-6*). As illustrated in Figure 2, the basic amine, methadone, rapidly disappears from the circulation and accumulates in the lung. Metabolites of methadone slowly appear in the perfusate. By using this technique, we found that the rabbit IPL metabolized methadone, chlorcyclizine, pentobarbital, and parathion but did not degrade imipramine (IMIP). However, all these chemicals, including imipramine, were degraded by lung homogenates or subcellular fractions, indicating important differences between metabolism *in vitro* and metabolism by IPL. Also, basic amines were found to accumulate in lung tissue with high tissue-to-blood ratios. By examination of the steady-state accumulation, we found that several mechanism(s) were responsible for the accumulation of basic amines in lung tissue (*4,5*).

The single pass or nonrecirculating IPL is useful for examining the removal, metabolism, and release of chemicals by the lung. We have used these techniques to examine the accumulation of basic amines by the lung. The lung can be perfused with a constant concentration of a chemical and then the efflux of that accumulated chemical from the lung examined. Using this procedure we studied the accumulation of the basic amine (imipramine and methadone) by the lung. The rate of efflux of accumulated imipramine (Fig. 3) was re-

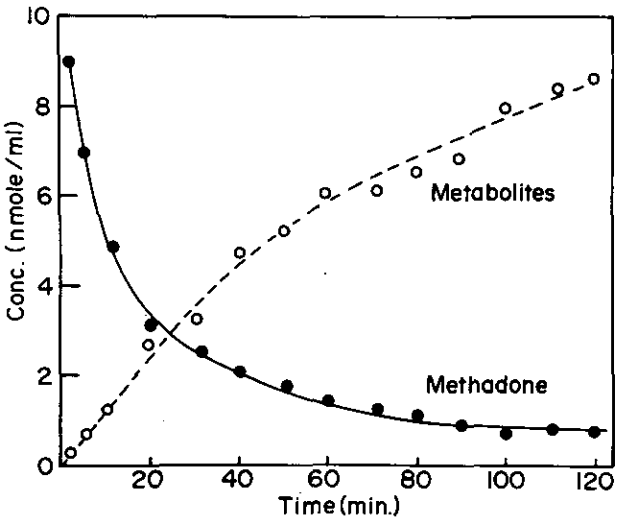

FIGURE 2. Disappearance of methadone and appearance of metabolites in perfusate of isolated perfused lung: (•) concentration of methadone: (O) concentration of metabolites, both expressed as nmole/ml of perfusate. Methadone (6 μmole) was added to 120 ml of perfusate. At the end of 120 min the lung contained approximately 26 nmole methadone/g of lung and 24 nmole metabolites/g of lung.

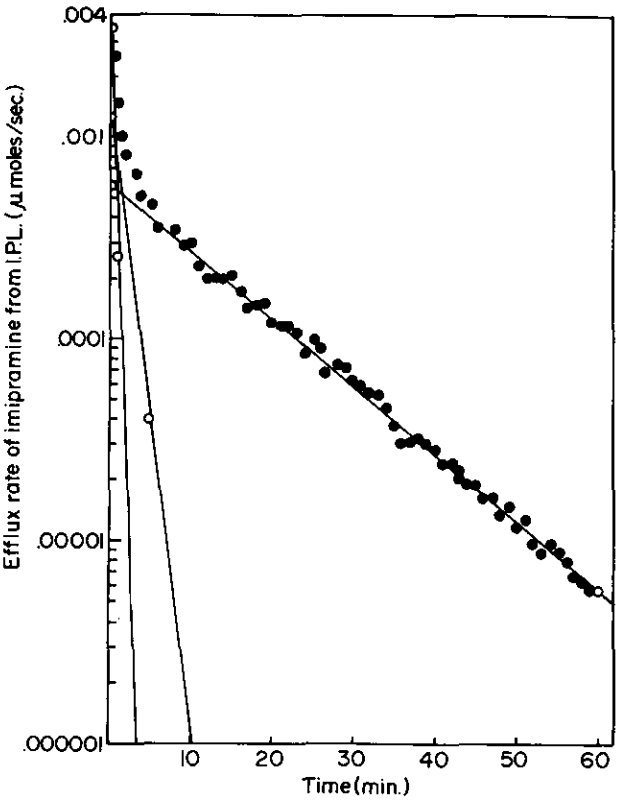

FIGURE 3. Variation with time of the efflux rate of ^{14}C-imipramine from the isolated perfused rabbit lung. Efflux rate (on a logarithmic scale) (μmole/sec) is plotted against the time after infusion of ^{14}C-imipramine into the pulmonary artery at a concentration of 0.0048 μmole/ml. The solid lines represent the exponential components obtained by nonlinear regression analysis.

solved into a series of exponentials indicating various pool or binding sites for the IMIP. Each of these pools, with its half-life, is in part responsible for the high accumulation of IMIP found in the lung. Further examination of these efflux rates indicated a pool with a half-life in excess of 5 hr and that the amount of imipramine remaining in the lung appeared to approach a constant amount (Fig. 4). The pool (or pools) did not result from irreversible binding and appeared to be associated with the phospholipid in the lung. We think that this pool is responsible for the persistence of imipramine and other basic amines in lung tissue and may be associated with drug-induced phospholipidosis (*7*).

Single-pass IPL preparations are also useful in examining the mechanism responsible for the removal of chemicals from the circulation by the lung. We have used these methods for examining the detoxification of prostaglandins by the lung.

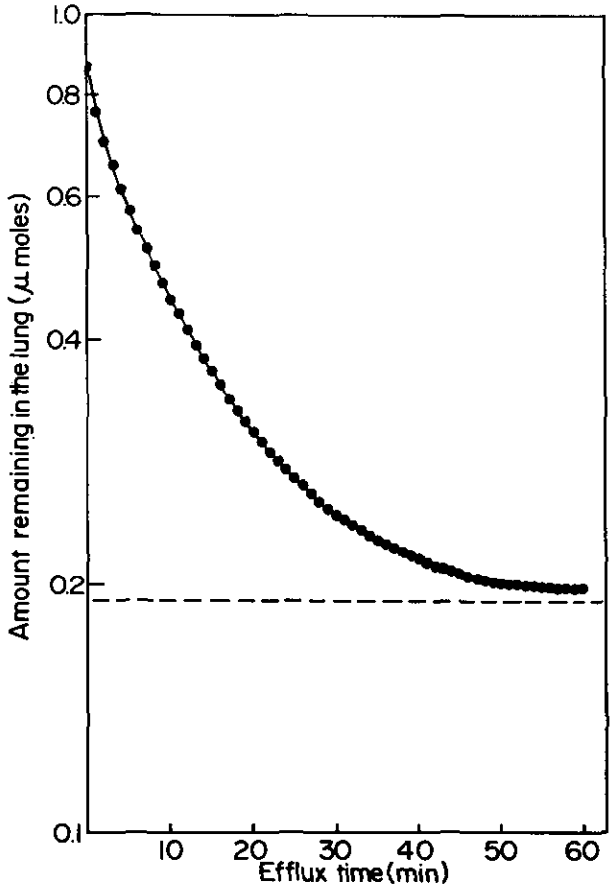

FIGURE 4. Time dependence of imipramine efflux from the isolated perfused rabbit lung. Lungs were infused with imipramine at a concentration of 0.0048 μmole/ml for 8 min, and the efflux of accumulated imipramine examined after cessation of the infusion. The amount of ^{14}C-imipramine in the lung is plotted against the time of efflux.

It has been established that the lung extensively degrades circulating prostaglandins (*8*). The enzymes responsible for this PG metabolism are found in the 100,000 *g* supernatant fraction of lung and thus are presumably found in the cytosol. Removal of PG was investigated by bolus injections of ^{3}H-PGF$_{2\alpha}$ and the vascular marker ^{14}C-dextran into the pulmonary artery of the isolated perfused lung. ^{3}H-PGF$_{2\alpha}$ metabolites appeared in the venous effluent at a later time than than the ^{14}C-dextran (Fig. 5), which indicated a rapid uptake and release of PG by the lung. ^{3}H-PGB$_1$ or ^{3}H-PGA$_1$ was also injected as a bolus with the vascular marker ^{14}C-dextran. These prostaglandins appeared with the ^{14}C-dextran in the effluent and no metabolites were detected, indicating little or no removal of these PG by the lung. In contrast, PGE$_1$, PGF$_{2\alpha}$, and PGA$_1$ were degraded *in vitro* by the 100,00*g* supernatant. This selective degradation of PG by the lung *in vivo* indicates selective removal of the PG from the circulation. These data imply that PGE$_1$ and PGF$_{2\alpha}$ are removed from the circulation by a transport system rather than by diffusion or binding.

To establish the existence of a pulmonary transport for PG, it is necessary to examine the unidirectional flux of the PG into the lung tissue. Rat lungs were perfused with either ^{3}H-PGE$_1$ or ^{3}H-PGF$_{2\alpha}$ and the vascular marker ^{14}C-dextran at a final concentration and flow rate. By measuring the radioactivity in the effluent one can calculate the net rate of flux of radioactivity into the lung, as seen in Figure 6. After subtraction of the dextran dilution into the vascular space the rate of PG flux from the perfusate into the lung was estimated by extrapolation to zero time indicated

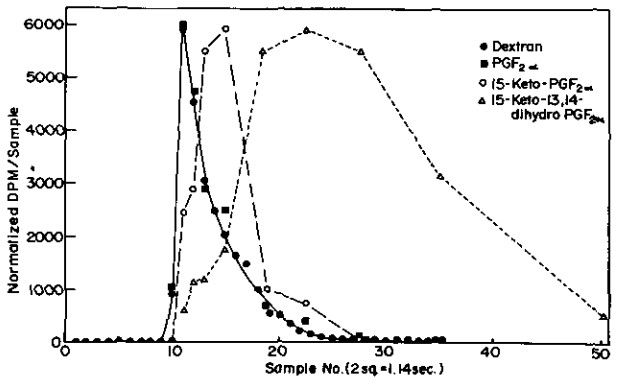

FIGURE 5. Appearance of ^{14}C-dextran, ^{3}H-PGF$_{2\alpha}$, and metabolites in the venous effluent after a bolus injection into the pulmonary artery of IPL.

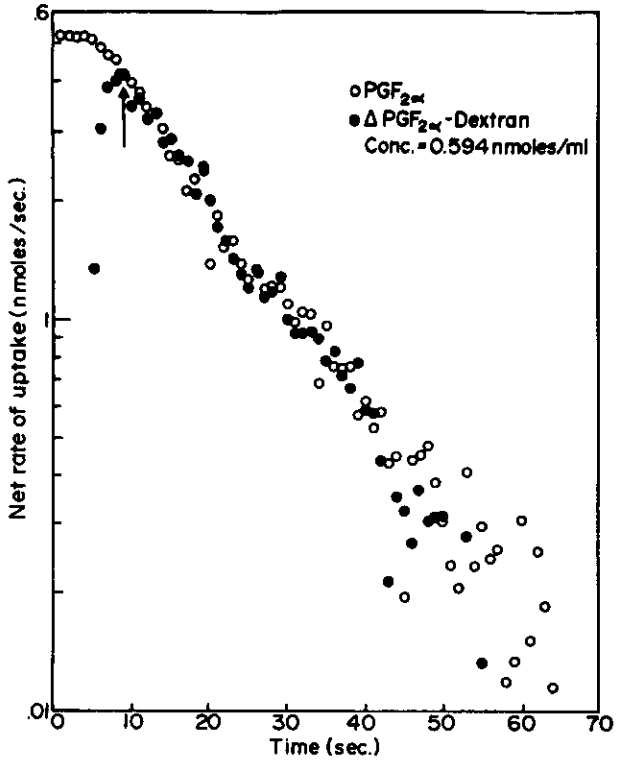

FIGURE 6. Variation with time of the net removal rate of radioactivity in IPL during a constant perfusion with ^{3}H-$PGF_{2\alpha}$ and ^{14}C-dextran. The lung was perfused at 30 ml/min with ^{3}H-$PGF_{2\alpha}$ and ^{14}C-dextran at a level of 0.594 nmole/ml: (•) ^{3}H-$PGF_{2\alpha}$ and metabolites; (O) difference between the ^{14}C-dextran and ^{3}H-$PGF_{2\alpha}$ influx velocities. The arrow denotes the time when the difference in the dextran and $PGF_{2\alpha}$ rates was at maximum.

by the arrow. At zero time, no PG has accumulated in the lung and no metabolism has occurred. The rate of PG flux was then investigated at various perfusate concentrations. The unidirectional flux of PGE_1 saturated with respect to supply rate (Fig. 7). This hyperbolic relationship is consistent with the hypothesis that PGE_1 is removed from the circulation by a carrier-mediated transport process.

Thus we have shown that PGE_1, $PGF_{2\alpha}$, but not PGA_1 nor PGB_1, were accumulated in the lung by the carrier-mediated process. The presence of a second unlabeled PG in the perfusate inhibited both the metabolism and uptake of a labeled PG (Fig. 7). The uptake of PGE_1 in the presence of 5 nmole/ml of PGE_2 was reduced. The V_{max} for PGE_1 was unaltered by PGE_2 while the K_m of PGE_1 increased. Thus, PGE_2 was a competitive inhibitor of PGE_1. This again is consistent with the hypothesis that PGs are accumulated in lung by a carrier-mediated transport process.

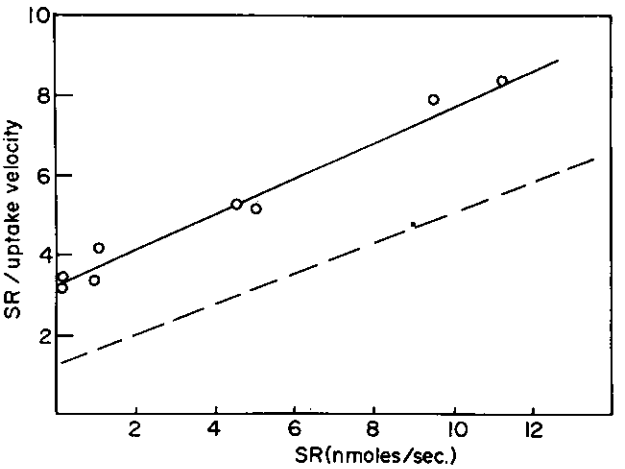

FIGURE 7. Dependence of the uptake velocity of PGE_1 into the IPL on the supply rate. Data plotted in the form of a Woolf plot of (ordinate) vs. the ratio of the supply rate to the uptake velocity; the supply rate (nmole/sec) (abscissa): the dashed line is uptake velocity in the absence of PGE_2; the solid line is uptake velocity in the presence of 5 nmole PGE_2/ml.

Thus, the inactivation system for PG in rat lung consists of a transport system and subsequent enzymatic inactivation. The relative effectiveness of these systems is shown in Figure 8. At a low supply rate, approximately 80% of PGE_1 was removed (into lung) and inactivated. As the supply rate increased, the percentage removal of PGE_1 slowly decreased to 20%. However, the percentage degradation of PGE_1 decreased very rapidly with increasing supply rate, and this removed, but unmetabolized PGE_1, returned to the circulation. Thus, at low concentrations the transport system is rate limiting while at higher concentrations the enzymatic inactivation becomes rate-limiting.

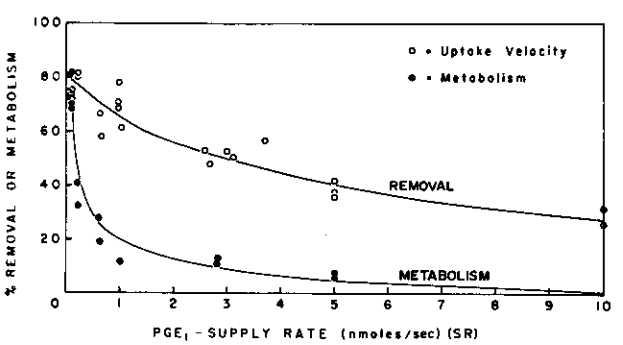

FIGURE 8. Effect of supply rate on the percentage removal and metabolism of PGE_1 by IPL. The ordinate shows percentage removal of metabolism; the abscissa gives supply rate (nmoles/sec).

In conclusion, we have developed and used an isolated perfused lung system that allows examination of the uptake, accumulation, metabolism and release of chemicals by the lung. Studies on some aspects of this problem would be difficult or impossible without the IPL preparation. We think that the system is highly suitable for the following: studies on the transport and metabolism of chemicals by lung; examination of accumulation of chemicals by lung; studies on metabolism of inhaled as well as circulating substances; investigation of the release of substances by the lung into the circulation or studies of the endocrine function of the lung. We believe that the IPL is a valuable tool in examination of these nonrespiratory lung functions.

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